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Prior weakening of *Macrophomina phaseolina* and *Fusarium* propagules for enhancing efficiency of *Brassica* amendments

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ABSTRACT

In a two year field study, the effect of varying intensities of sub-lethal heating on the efficiency of *Brassica* amendments in controlling viable populations of *Macrophomina phaseolina* and *Fusarium oxysporum* f sp. *cumini* was determined in an arid region of India. After 30 d of dry summer exposure of pathogen infested soil, incorporation of mustard residues and oil cake (0.18% and 0.04% w/w) and then applying one irrigation caused significant reduction by 75.3–81.3% in viable counts of *M. phaseolina* that causes dry root rot of legumes and by 93.9% in counts of *F.o.* f. sp. *cumini* causing wilt of cumin (*Cuminum cyminum* L.) at 0–15 and 16–30 cm depths. Increasing duration of summer exposure to 60 d improved the reductions in viable propagules of *M. phaseolina* by 83.6–90.4% and in *F.o.* f. sp. *cumini* by 78.2–94.8% at same soil depths. At certain heat levels, reduction in viable population of *Fusarium* due to amendments and irrigation was greater than that recorded in *Macrophomina.* Significantly low levels of reduction in pathogenic propagules of *Macrophomina* (63.9–71.4%) and *Fusarium* (48.0–57.2%) under shade compared to unshaded conditions indicated that mild heating did not cause discernible weakening effect. In second season also, 89.2–91.5% and 78.5–95.8% reduction in counts of *Macrophomina* and *Fusarium*, respectively was achieved by the application of amendments after 60 d of summer exposure at 0–30 cm soil depth. These results suggested a new approach to improve the control of soil-borne plant pathogens in hot arid regions by combining prolonged sub-lethal heating, effective naturally available on-farm wastes as soil amendments and one summer irrigation.

1. Introduction

Macrophomina phaseolina (Tassi) Goid. and *Fusarium oxysporum* f.sp. *cumini* Prasad and Patel (*Foc*), are two important soil-borne plant pathogens in arid regions of India. Under rainfed cultivation, concurrent heat and moisture stress favour development of *Macrophomina* induced charcoal rot on guar (*Cyamopsis tetragonoloba* (L.) Taub.), cowpea (*Vigna unguiculata* (L.) Walp.) and several other legumes grown for vegetable and seed purposes, while in irrigated pockets of the region *Foc* causes a severe wilt on a seed spice, cumin (*Cuminum cyminum* L.) cultivated during the winter season (Lodha et al., 1986). The population density of these pathogens increases in the soil with each year of cultivation of susceptible crops and the inoculum density are directly proportional to the disease intensity in the field (Lodha, 1995). Soil solarization has been effective in reducing the population density of these pathogens due to ample availability of high temperatures and solar irradiations during crop-free periods (Lodha, 1995). However, lethal levels of temperatures achieved under polyethylene mulching have been speculated to be detrimental to beneficial soil microflora (Katan et al., 1992). Therefore, there is increasing interest in ascertaining the effect of sub-lethal heating on pathogen viability and in the possibility of reducing pathogen populations without drastic heating (Assaraf et al., 2002). The pathogen control can also be improved by integrating other effective management strategies. Thus, combining cruciferous residues with one irrigation during hot summer months (April–June) has been developed as an alternative approach for achieving reasonable control of both the target soil-borne plant pathogens in the region (Mawar and Lodha, 2002; Israel et al., 2005).

Like other workers (Freeman and Katan, 1988; Arora et al., 1996), we also hypothesized that if pathogenic propagules are exposed to a sub – lethal dose of heat, the stressed individuals may be weakened and more vulnerable to additional management strategies. The present investigation deals with effect of different levels of sub-lethal heating, *Brassica* amendments and summer irrigation on the survival of *M. phaseolina* and *Foc* propagules in a hot arid environment.

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2. Materials and methods

2.1. Experimental site

The experiments were conducted at the Central Arid Zone Research Institute, Jodhpur, Rajasthan, India during summer (April–June) of 1998 and 1999. The loamy sand soil of the experimental site had sand 85%, clay 8.9%, silt 5.5%, nitrogen 0.031%, organic matter 0.43%, Olsen P 7 $\mu g\, g^{-1}$ pH 8.1 and electrical conductivity (soil:-water ratio 1:2.5) 0.088 d Sm^{-1}

2.2. Inoculum preparation

Virulent pathogenic strains of *Foc* isolated from the diseased roots of cumin and *M. phaseolina* isolated from guar were multiplied separately in bulk on 5% corn meal: sand medium for 15 d at 30 ± 2 °C. The spores (conidia and chlamydospores) of *Foc* and sclerotia of *M. phaseolina* so produced were passed separately through a 300-mesh (53 μm) sieve (Papavizas and Klag, 1975). The infested material left on the sieve was first examined under the microscope to confirm that it contains only chlamydospores of *Fusarium* or sclerotia of *M. phaseolina* and then mixed with several kg of field soil to prepare pathogen infested soil. This soil was then left for 7–10 d in bright sunlight (37–41 °C) for further stabilization before use. The viable populations of *Foc* and *M. phaseolina* were determined on their respective selective media described in section 'biological assays.'

2.3. Experimental design

The experiment in the 1998 season was arranged to study the effect of four intensities of heat in four sets (A–D) of 30×30 cm pits. *Macrophomina* and *Foc* propagules in soil were separately exposed to natural dry heating for 30 (A – May) and 60 (B – June) and 0 d (C – Infested soil kept in laboratory, was exposed from June 1 under open field) and for 60 d (D – June) under shaded conditions (Fig. 1). Three sets (A–C), each comprised of six pits arranged in split–split plot design (1 m apart) were dug in 4×3 m plots in an open field receiving bright sunlight for at least 10–11 h d^{-1}. Pits for set D were dug under a dense canopy of trees 50 m away from other pits. Three plots comprised three replications for each set. Thus, there were 12 pits for each set. On March 31, all the pits of A, B and D were filled with 9 kg of pathogen infested soil prepared from the aforesaid procedure. Two 5 g subsamples were taken soon after from each pit for the estimation of viable propagules of both pathogens. Infested soil of set C was kept separately in 12 polyethylene bags of 9 kg capacity at room temperature (30–35 °C) in the laboratory. The soil in pits of set A was taken out and separately amended on April 30 with 1). ground up residues of Indian mustard (*Brassica juncea* (L.) Czern. and Coss) – MR (0.18%w/w) and 2). MR + Mustard oil cake–MC (0.04% w/w) and then refilled in randomly selected pits. Pits filled only with *Macrophomina or Foc* infested soil served as 3). Wet (Summer irrigation – SI) and 4). Dry (DS) non-amended controls. On May 1, one irrigation of 45 cm depth was applied by flooding to bring the soil to field capacity (10.4% w/w or –0.003 Mpa) in plots having pits of set A except the dry control, which were separated by a thick soil bund to check water flow. Infested soil from set B and D was withdrawn from pits on May 31 and on the same day polyethylene bags containing pathogen infested soil of set C kept in the laboratory was brought to the field. Pathogen infested soil of sets B, C and D was amended separately with aforesaid combinations of *Brassica* residues except that of the wet control (SI). Irrigation was applied on June 1 in all the sets (B, C and D) in the manner described above.

In 1999, experimental details remained same except that set A (May – 30 d) was not included. Thus, the experiment had three levels of heat viz., A – June unshaded with 60 d summer exposure, B – brought from room temperature in June and C – shaded.

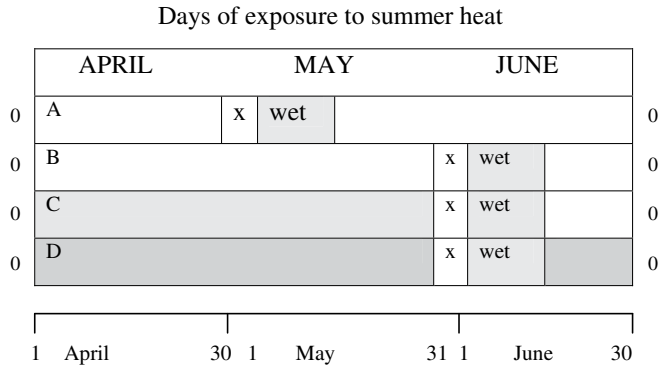

Fig. 1. Schematic plan of the experiment showing varying intensities of sub-lethal heating of *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *cumini* propagules in soil achieved by different days of summer exposure before applying amendments and irrigation. A – 30 days and B – 60 days of summer exposure, C – 60 days at room temperature before summer exposure and D – under shade: 0 – sampling date: x – application of amendment and irrigation.

2.4. Soil temperature and moisture

Soil temperatures were recorded at 15 cm depth in one representative pit of each treatment of all the sets from the second day of irrigation by inserting soil thermometers in order to take mean values of 0–15 and 16–30 cm depth. Two soil samples were collected at depths of 0–15 and 15–30 cm by a tube auger (2.5 cm) at 08.00 h on July 1 during both years of the experiment. Half of each soil sample was used to determine soil moisture by a gravimetric method, while the remainder was used for estimating viable propagules of *M. phaseolina* or *Foc*.

2.5. Biological assays

Colony forming units (CFUs) of *M. phaseolina* were determined by sprinkling 50 mg of each soil sample on Chloroneb–Mercury–Rose bengal–Agar (CMRA) medium (Meyer et al., 1973) in 9 cm Petri dishes. The CFUs of *Foc* were estimated by a serial dilution technique on modified peptone – PCNB (664 mg l^{-1}) medium (Papavizas, 1967). A suspension of 10 g of soil in 90 ml of sterilized, deionized water was serially diluted two more times (10^3). One milliliter of the final dilution was pipetted onto one plate. When no CFUs were detected at 10^3, these were estimated at 10^2 dilutions. White restricted colonies of *Foc*, which later turned pinkish, were easily distinguishable from other *formae speciales* because of their distinct shape and size. The proportion of survival of *Macrophomina* or *Foc* propagules in each replication was computed by dividing the number of viable propagules after the completion of the experiment by the number of propagules at the beginning of the experiment and percentage reductions in viable propagules of both the pathogens were calculated for each treatment.

2.6. Statistical analysis

The percent reduction data of *Macrophomina* and *Foc* from each pit were transformed to angular values before analysis. The transformed data were analyzed following a split–split plot design to identify the significance of main effects of heat levels, amendments,